

## **Elevated Skin Blood Flow Influences Near Infrared Spectroscopy Measurements During Supine Rest**

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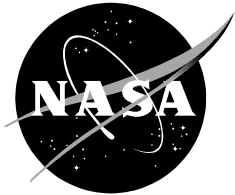
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## Summaries

### Abstract

Near infrared spectroscopy (NIRS) is a non-invasive technique that allows determination of tissue oxygenation/blood flow based on spectro-photometric quantitation of oxy- and deoxyhemoglobin present within a tissue. This technique has gained acceptance as a means of detecting and quantifying changes in tissue blood flow due to physiological perturbation, such as that which is elicited in skeletal muscle during exercise. Since the NIRS technique requires light to penetrate the skin and subcutaneous fat in order to reach the muscle of interest, changes in skin blood flow (SBF) may alter the NIRS signal in a fashion unrelated to blood flow in the muscle of interest. The aim of this study was to determine the contribution of SBF to the NIRS signal obtained from resting vastus lateralis muscle of the thigh.

### Methods

Seven subjects rested supine with the vastus lateralis instrumented for simultaneous continuous measurement of SBF and NIRS. SBF was determined by laser Doppler velocimetry, and muscle oxygenation (StO<sub>2</sub>) and hematocrit (Hct) were determined using NIRS. In order to increase SBF in a defined manner, local heating was applied to the skin area surrounding the NIRS probe utilizing a heating jacket containing circulating water across a range of defined temperatures (i.e. 32-42 °C). Laser Doppler- and NIRS-derived variables at four steady state temperatures were averaged and compared to baseline using repeated measures ANOVA. Pearson's Product Moment Correlations were employed to determine whether a significant relationship existed between laser Doppler- and NIRS-derived variables.

### Results

SBF and Hct were significantly elevated from baseline at the highest value for local heating (i.e. 42°C); StO<sub>2</sub> was significantly elevated during both the two highest (i.e. 40 and 42°C) values for local heating. Also, there were significant relationships between the percent change in SBF and StO<sub>2</sub> and between SBF and Hct when expressed as a percentage of the total response.

### Conclusions

Results from this study indicate that a significant portion of the NIRS signal collected in the resting *vastus lateralis* muscle can be attributed to local skin temperature-dependent SBF. Therefore, interpretation of NIRS skeletal muscle data from investigations that result in elevated SBF, such as prolonged exercise or heating, should be viewed with caution.

## Introduction

The measurement of muscle blood flow and oxygenation are important to studies of normal and disease states. Understanding the determinants of muscular performance and the proportional influences of central and peripheral adaptations both at rest and during exercise may provide important clues regarding functional limitations. From such knowledge, appropriate training or therapeutic modalities can be developed for acute injuries, chronic diseases, and space flight, as well as for the physical training of athletes and the general population.

Invasive techniques provide the most direct assessment of muscle blood flow and oxygen delivery and have been used to assess the validity of non-invasive methodologies. Classically, muscle blood flow has been determined by either dilution (i.e. thermo-dilution) or clearance (i.e. microspheres, xenon clearance) techniques. However, the application of these relatively complex techniques in experimental protocols that involve prolonged or vigorous exercise has significant practical limitations. In addition, both plethysmography and Doppler flow measurements may be limited in their experimental utility because they are unable to distinguish between blood flow to working muscle, non-working muscle, and other tissues [Wilson, 1989] and are also incapable of providing data on actual oxygen delivery and utilization [McCully, 2000].

Near-infrared spectroscopy (NIRS) is a relatively new technique that has gained acceptance as a non-invasive method to measure oxygen saturation and blood flow parameters. Early studies in the use of light to assess skeletal muscles were performed by Chance and colleagues [Chance, 1959; Chance, 1963] beginning in the 1950's, but more recently, NIRS has been used to assess and monitor patients in trauma cases and surgery [Hampton, 1986], especially those involving the brain [van Beekvelt, 2001].

Both physicians and physiologists have employed NIRS to monitor blood flow and tissue oxygenation of skeletal muscle [McCully, 2000]. NIRS uses a probe containing both a light source and a detector placed on the skin above the region of interest. Light from the source is directed through the skin and is scattered in all directions as it penetrates the tissue [Grassi, 1999]. The detector is placed a set distance from the source and measures the small amount of light that is reflected in the pattern of a shallow arc from the light source [McCully, 2000]. The light is filtered, either at the light source or the detector, such that only defined wavelengths of light are measured, usually in the range of 700-900 nm. Hemoglobin and myoglobin are the primary absorbers of light in this range and the presence of oxygen alters the wavelength that is absorbed by the heme groups in these compounds [McCully, 2000]. The amount of returning light within each wavelength is proportional to the concentrations of hemoglobin and myoglobin in their oxygenated or deoxygenated states. One of the primary advantages of this technique over existing methods is that continuous monitoring of muscle blood volume and oxygenation is possible.

The relationship between absorption of light and concentration is described by the Beer-Lambert Law, modified for scattering media:

$$A = \epsilon [c] LB + G$$



where  $A$  is the absorption of light,  $\epsilon$  is the extinction coefficient for the chromophore,  $[c]$  is the chromophore concentration,  $L$  is the distance between the light source and the detector,  $B$  is the path-length resulting from the scattering of light within the tissue, and  $G$  is a “lump” parameter related to tissue and optode geometry. Path-length and geometry can be difficult to measure, but are assumed to be relatively constant when distance between the light source and detector are kept constant. The path-length can be estimated from time-of-flight analyses using pulses of light, but is variable with changes in absorption across subjects and measurement sites based upon the tissue being sampled. Because of these problems, absolute values for chromophore concentrations are difficult to determine accurately and NIRS parameters are usually expressed as relative values [Boushel, 2000].

Skin blood flow represents a potential confounding factor when using NIRS to assess muscle blood flow and oxygenation. Mancini, et al. [Mancini, 1994] observed that the signals obtained in the range of 760-800 nm were relatively stable despite changes in skin blood flow. Further, the authors concluded that the small volume of skin relative to the muscle would have little impact on NIRS measurements. Other authors [Hampson, 1986; Piantadosi, 1986], using data derived from an animal model, have suggested that the skin overlying the muscle of interest contributes less than 5% of the signal. However, Chuang, et al. [Chuang, 2002] observed a recovery in NIRS-derived oxygen saturation levels during exercise, despite no change in directly measured venous blood saturation, and attributed the changes to an increase in skin blood flow. These investigators and others [Maehara, 1997] were able to attenuate or eliminate this apparent recovery of oxygen saturation detected by NIRS by inducing skin vasodilation prior to exercise, either by pre-warming the skin or by the administration of capsaicin. These data indicate that vasodilation of the skin vasculature overlying the muscle of interest may have a more significant impact on overall NIRS signal than previously thought.

The purpose of this project was to evaluate the relationship between skin and muscle blood flow parameters at rest during the manipulation of the skin temperature overlying the muscle of interest. It has been well established that changes in skin temperature illicit an increase in skin blood flow [Johnson, 1996] with no change in blood flow of the underlying muscle tissue [Johnson, 1976]. We hypothesized that elevation of skin temperature would result in an increased skin blood flow with no commensurate changes in NIRS-derived blood flow indices from the underlying muscle tissue.

## **METHODS**

### **Overall protocol**

This project was completed in two phases. The purpose of the first phase was to verify skin temperature could be manipulated successfully using a bladder placed over the thigh through which water was circulated at defined temperatures. In the second phase, a similar protocol was performed except that indices of skin blood flow (determined using laser Doppler velocimetry; LDV) and muscle blood flow (determined using near infrared spectroscopy; NIRS) were recorded continuously throughout the experiment.

Two subjects participated in the first phase of this project, and seven subjects participated in the second. The procedures for this investigation were reviewed and approved by the NASA-Johnson Space Center Committee for the Protection of Human Subjects. Subjects reported to the laboratory rested and had not performed any exercise on the day of testing. Subjects were dressed in comfortable shorts that were not constrictive and allowed easy access to the thigh muscle to be tested.

### **Temperatures at site of measurement during local heating**

Two subjects participated in a protocol to verify skin temperature over the vastus lateralis muscle could be manipulated at the LDV and NIRS measurement sites. Subjects were supine for a period of one hour. During the first 30 minutes, subjects were instrumented for the measurement of skin temperature at two sites on the right thigh (near the mid-point of the vastus lateralis muscle). These sites corresponded to the LDV and NIRS measurement sites to be used during the second phase of the study. The thermistors were adhered with a double-sided adhesive disk with the thermistors' sensing surface against the skin. The holders for the LDV and NIRS probes were placed on top of the skin temperature thermistors. The probes were placed to simulate the microenvironment between the probes and the skin (to mimic the measurement protocol used in the second phase of this project). A water-filled bladder was placed around the skin temperature thermistors and probe holders. All air was evacuated from the bladder prior to testing and water was circulated through the bladder for at least five minutes before measurements began. Water flow was maintained at approximately  $1 \text{ liter} \cdot \text{min}^{-1}$  throughout all experiments. Water temperature within the heating bladder was manipulated using a circulating water bath<sup>1</sup>, and water was circulated through the bladder via Tygon tubing using the water bath's pump. Flow rate and bladder water pressure were controlled using a screw clamp on the outflow side of the bladder. The outflow of the bladder was emptied back into the water bath for recirculation.

During the pre-testing phase and the first 5 minutes of measurements, the water bath temperature was maintained at  $30^{\circ} \text{ C}$  ( $86^{\circ} \text{ F}$ ). Thereafter, the water bath temperature was increased to  $36^{\circ} \text{ C}$  ( $96.8^{\circ} \text{ F}$ ) then to  $42^{\circ} \text{ C}$  ( $107.6^{\circ} \text{ F}$ ), and held constant for five minutes at each temperature. These temperatures were well tolerated by the subjects and were chosen to elicit a range of skin blood flow levels.

As a result of observations made in the first phase of the study, two important modifications were made to the local skin heating protocol. First, the observed skin temperatures at the measurement sites were consistently lower than the water bath temperature, possibly due to significant heat loss during the transfer of water through the Tygon tubing into the bladder. Therefore, water bath temperature was increased to approximately  $45^{\circ} \text{ C}$  such that local skin temperature during the procedure increased to approximately  $42^{\circ} \text{ C}$ . Secondly, subjects were covered with a single layer of towels except for the head and at the site of the water bladder. Previous investigations have shown that skin blood flow responses are altered when ambient temperatures are different from those at the site of interest [Wenger, 1985]. In this manner, the effect of room temperature on skin blood flow responses to local heating was minimized.

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<sup>1</sup> Precision Scientific, Model 260, Winchester, Va.

## Changes in LDV and NIRS with local heating

In the second phase of this project, seven subjects participated in a similar local heating protocol as employed in the first phase with the addition of LDV and NIRS measurements. Subjects were supine for a period of 1 hour. During the first 30 minutes, subjects were instrumented for the LDV and NIRS measurements as well as skin temperature (thermistors) near the site of measurements. One skin temperature thermistor was placed adjacent to LDV measurement site, and one was placed adjacent to the NIRS measurement site. A water-filled bladder was placed over these sensors, and water was circulated through it to control skin temperature in a manner identical to that described above. During the pre-testing phase and the first 5 minutes of measurements, the water bath temperature was maintained at 30 °C. Thereafter, the water bath temperature was increased to 36 °C, 42 °C, and 45 °C and held constant for five minutes at each temperature. LDV, NIRS indices, and skin temperatures were recorded continuously throughout the protocol.

Laser Doppler indices were measured using an integrated probe<sup>2</sup> interfaced with a master control unit<sup>3</sup>. The laser Doppler system was calibrated prior to use in the standard manner with a zeroing disk and motility standard. Skin temperature was measured with thermistors<sup>4</sup> interfaced with a temperature controller<sup>5</sup>. Skin temperature thermistors were calibrated prior to use at 30, 36, and 42 °C using a certified thermometer. LDV and thermistor data were routed through an A-D converter<sup>6</sup> and saved using the vendor-provided software<sup>7</sup>. Laser Doppler-derived variables included perfusion, concentration of moving blood cells (CMBC; an index of tissue hematocrit), and velocity. CMBC was derived from the total light received back from the integrating probe as a result of striking a red blood cell. Velocity was calculated from the mean of the Doppler shifts observed in the returning light, and perfusion was calculated from the product of velocity and CMBC. Each parameter was expressed in arbitrary units.

NIRS measurements were collected using a tissue spectrometer<sup>8</sup> interfaced with a standard 25 mm probe<sup>9</sup>. The tissue spectrometer was calibrated prior to each test using the vendor-provided calibrator and calibration procedures. Calibration was verified with the standards provided by the manufacturer<sup>10</sup>. Data were collected at 3-second intervals with a research and developmental version of the vendor-provided software<sup>11</sup>. Oxygen saturation (StO<sub>2</sub>), hematocrit (Hct), total hemoglobin (HbT), oxyhemoglobin, and deoxyhemoglobin were derived. Oxygen saturation and hematocrit are assumed to reflect measured values, but hemoglobin values were expressed in arbitrary units. Total hemoglobin and hematocrit are thought to provide an estimation of blood volume in the tissue [Mancini, 1994], and thus tissue blood flow.

Immediately following the protocol, skinfold thickness at the site of the NIRS probe was measured in triplicate using skinfold calipers. The mean of the three measurements was accepted

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<sup>2</sup> PF413 Integrating Probe, Perimed, Inc., Jarfalla, Sweden

<sup>3</sup> PeriFlux 4001 Master, Perimed, Inc., Jarfalla, Sweden

<sup>4</sup> PF440 Temperature Sensor, Perimed, Inc., Jarfalla, Sweden

<sup>5</sup> PeriTemp 4005 Heater, Perimed, Inc., Jarfalla, Sweden

<sup>6</sup> PF472 Controller Box, Perimed, Inc., Jarfalla, Sweden

<sup>7</sup> Perisoft v5.10, Perimed, Inc., Jarfalla, Sweden

<sup>8</sup> InSpectra, Model 325, Hutchinson Technologies, Inc., Hutchinson, Minn.

<sup>9</sup> InSpectra, Hutchinson Technologies, Inc., Hutchinson, Minn.

<sup>10</sup> InSpectra System Check, Hutchinson Technologies, Inc., Hutchinson, Minn.

<sup>11</sup> Casper, Hutchinson Technologies, Inc., Hutchinson, Minn.

as representative for that subject. The mean skinfold was then divided by two to determine the amount of skin and subcutaneous fat overlying the muscle [Van Beekvelt, 2001].

## Data Analysis

During each procedure, concurrent event markers were made in each data file to signify the beginning and end of each steady-state temperature stage. All data were averaged post-test in 30-second intervals during each five-minute stage. LDV and NIRS data were available for all subjects, but skin temperature data were unavailable for one individual. Ten samples were available at each stage for all but one subject; in one subject, only nine measurements were available at each stage.

Mean data for each stage of the protocol were compared using a one-way ANOVA. Tukey's post-hoc test was used to determine where differences occurred when a significant main effect was observed. Mean data were represented as mean  $\pm$  standard error. Pearson's Product Moment Correlation was performed for the percent change from baseline in each LDV variable against the percent change from baseline in each NIRS variable to determine whether a significant relationship was present. All 30-second samples were used in these analyses. Additionally, for correlations across subjects LDV-derived measures were expressed as the percentage of the baseline observed at 30° C as well as the percentage of the total skin blood flow response.

## Results

In the first phase of this project, skin temperatures in the two test subjects (one male and one female) were observed to increase but were not well controlled by the water bladder (Table 1). At the 30° C water bath temperature the observed skin temperatures were higher, and at the 42° C water bath temperature the skin temperatures were lower than desired. As a result of these observations, the testing protocol for the second phase of this study was modified to control skin temperature rather than circulating water bath temperature.

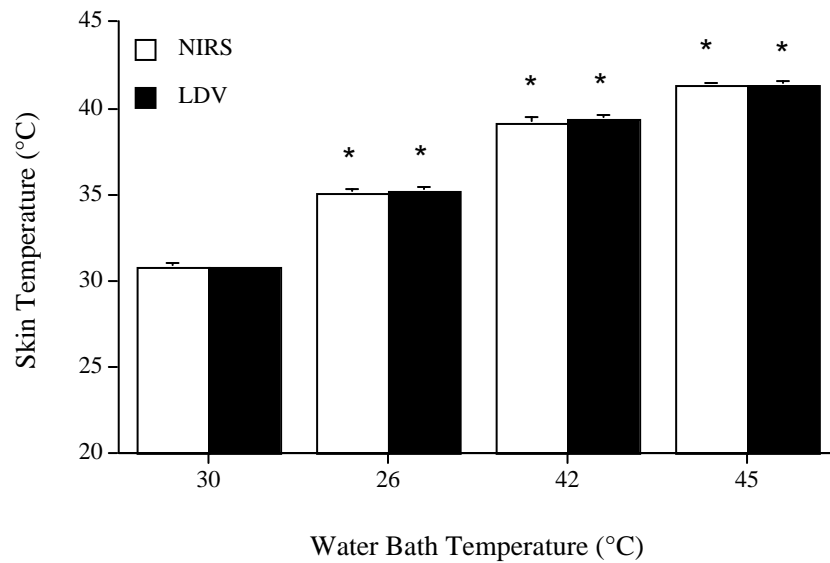
**Table 1. Skin temperature measured at the site of the LDV and NIRS probes during the first phase of the study.**

Water Bath (°C)	Subject 1		Subject 2	
	NIRS-Site (°C)	LDV-Site (°C)	NIRS-Site (°C)	LDV-Site (°C)
30.1	32.2	32.4	31.0	31.6
36.0	34.3	33.8	35.3	35.2
41.9	37.7	37.1	36.7	37.2

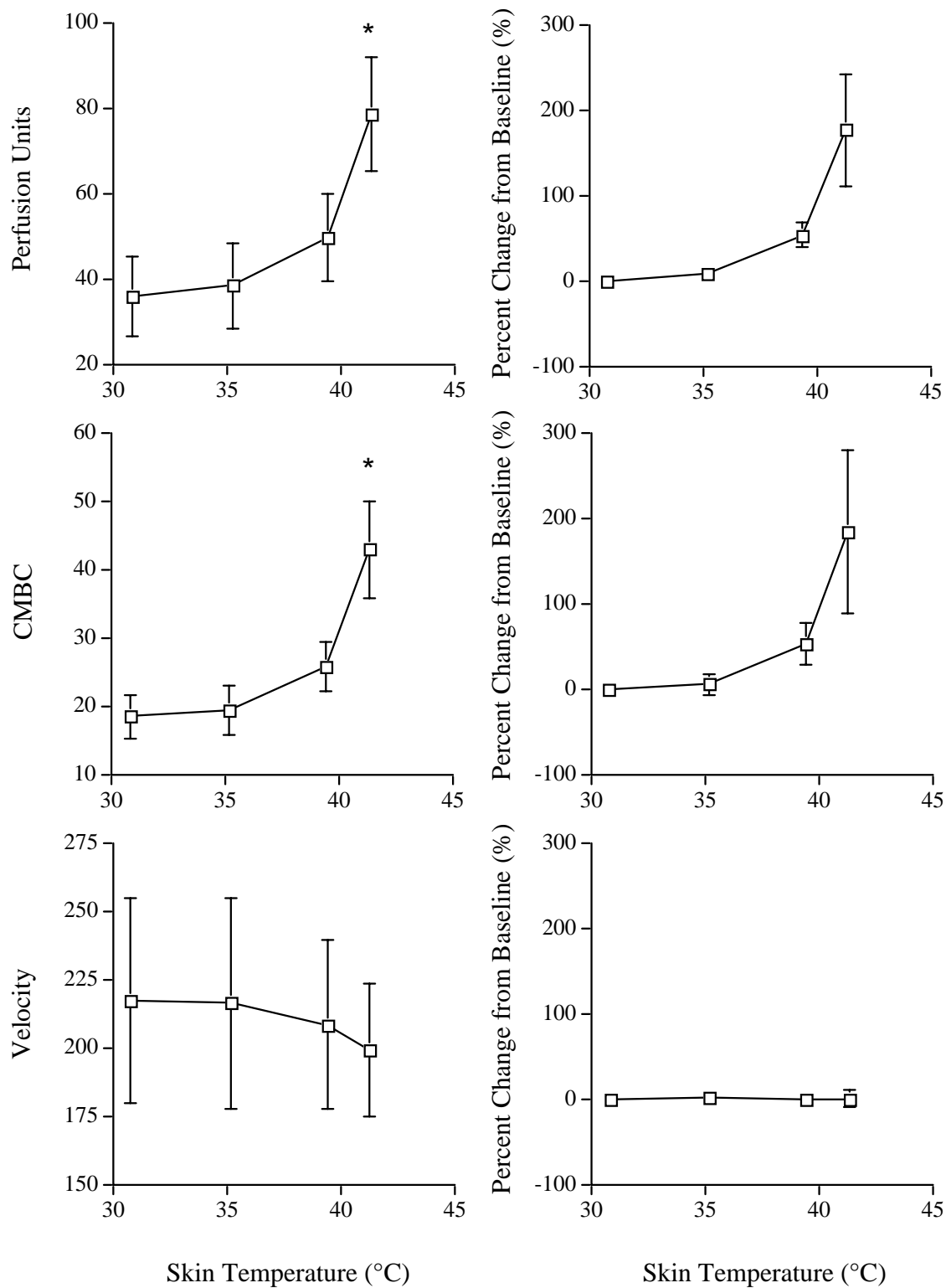
Six males and one female volunteered to participate in the second phase of the study. Subjects averaged (mean  $\pm$  standard deviation) 32  $\pm$  3 years old, 82.8  $\pm$  14.3 kg, and 178  $\pm$  8 cm tall. The

mean skin thickness at the site of measurement (one-half of the measured skinfold) was  $7.6 \pm 3.0$  mm (range: 3.9-12.1 mm).

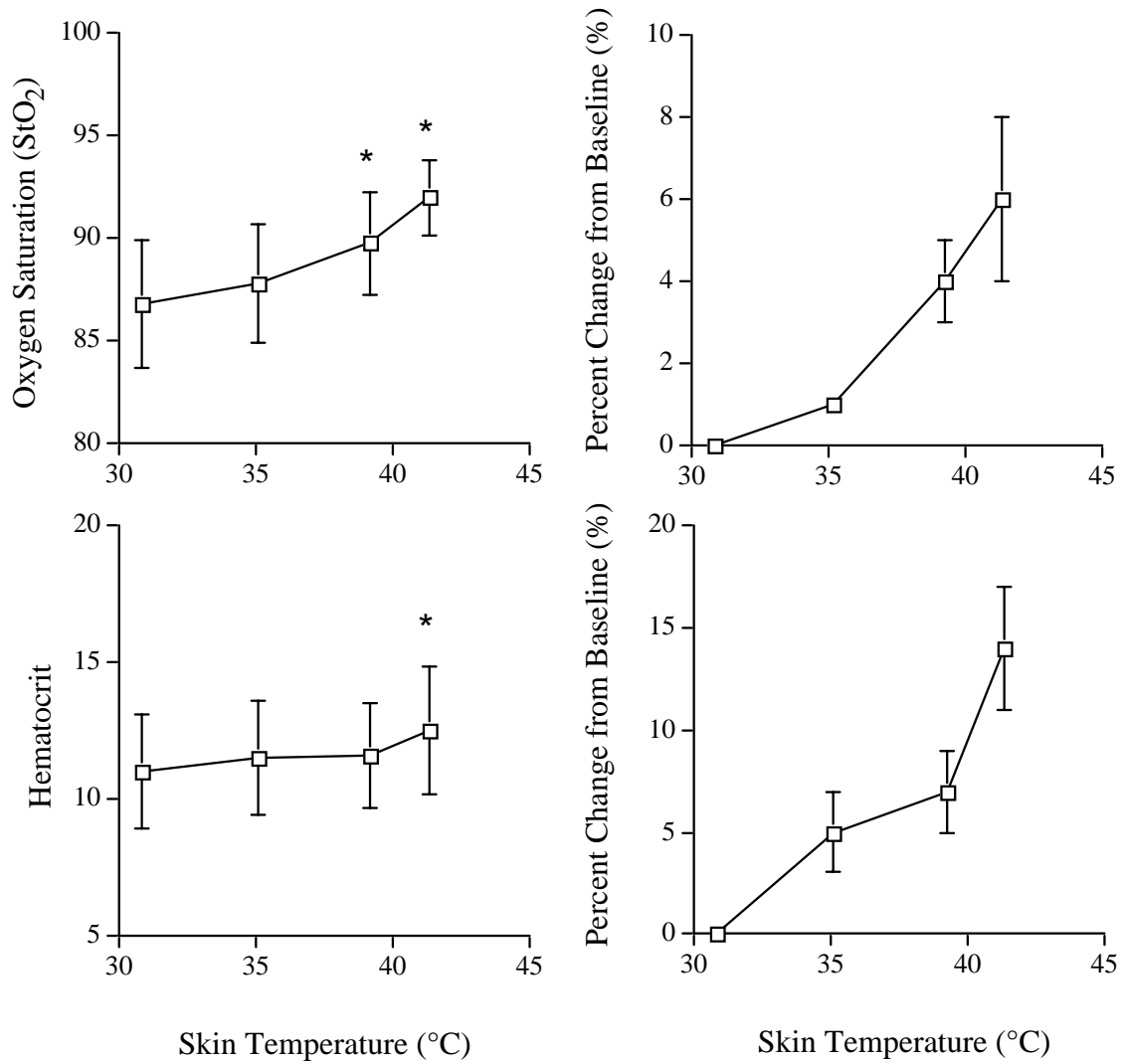
Local skin temperature near the site of the NIRS and LDV probes increased with each stage of the protocol (Figure 1). LDV indices of skin blood flow, perfusion and CMBC, were not different from baseline in the second and third stages of the protocol, but both increased significantly during the fourth stage (Figure 2); LDV-derived velocity of skin blood flow did not change at any stage, suggesting that the increased SBF was primarily the result of increased total volume of the capillaries carrying blood. NIRS-derived tissue oxygen saturation, hematocrit, total hemoglobin, and oxyhemoglobin were unchanged during the first two stages of the protocol, but significantly increased from baseline in the third and fourth stages (Figures 3 & 4). Deoxyhemoglobin decreased significantly from baseline in the third and fourth stages.



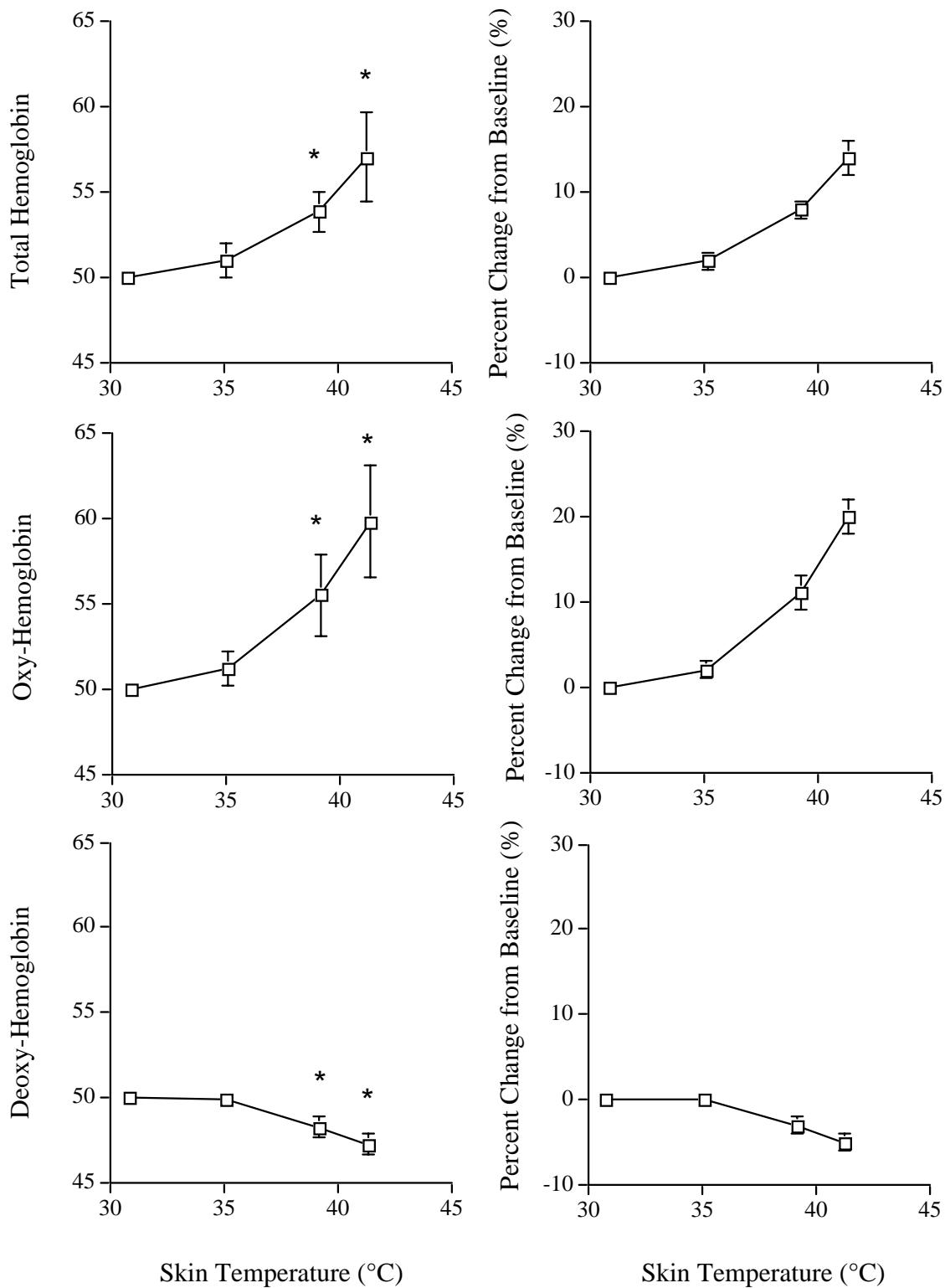
**Figure 1. Local skin temperature measured near the LDV and NIRS measurements in the second phase of this project (\*p<0.05)**



**Figure 2. Laser Doppler velocimetry measures during the local skin heating perturbation. (\*p<0.05)**



**Figure 3. NIRS-derived tissue oxygen saturation and hematocrit during the local heating procedure. \*p<0.05**



**Figure 4. NIRS-derived total hemoglobin, oxy-hemoglobin, and deoxy-hemoglobin during the local heating procedure. \*p<0.05.**



There was a significant relationship between LDV-derived perfusion, CMBC, and NIRS-derived variables in almost all cases (Table 2). The relationship between NIRS-derived oxygen saturation and CMBC when expressed as percent change relative to baseline was not significant. In almost all cases, the relationships between these variables across subjects were improved when the LDV-derived variables were normalized across subjects by expressing them as a percentage of the total response within individual subjects (Figure 5).

**Table 2. Correlations between LDV-derived variables and NIRS-derived variables across subjects. \*p<0.05**

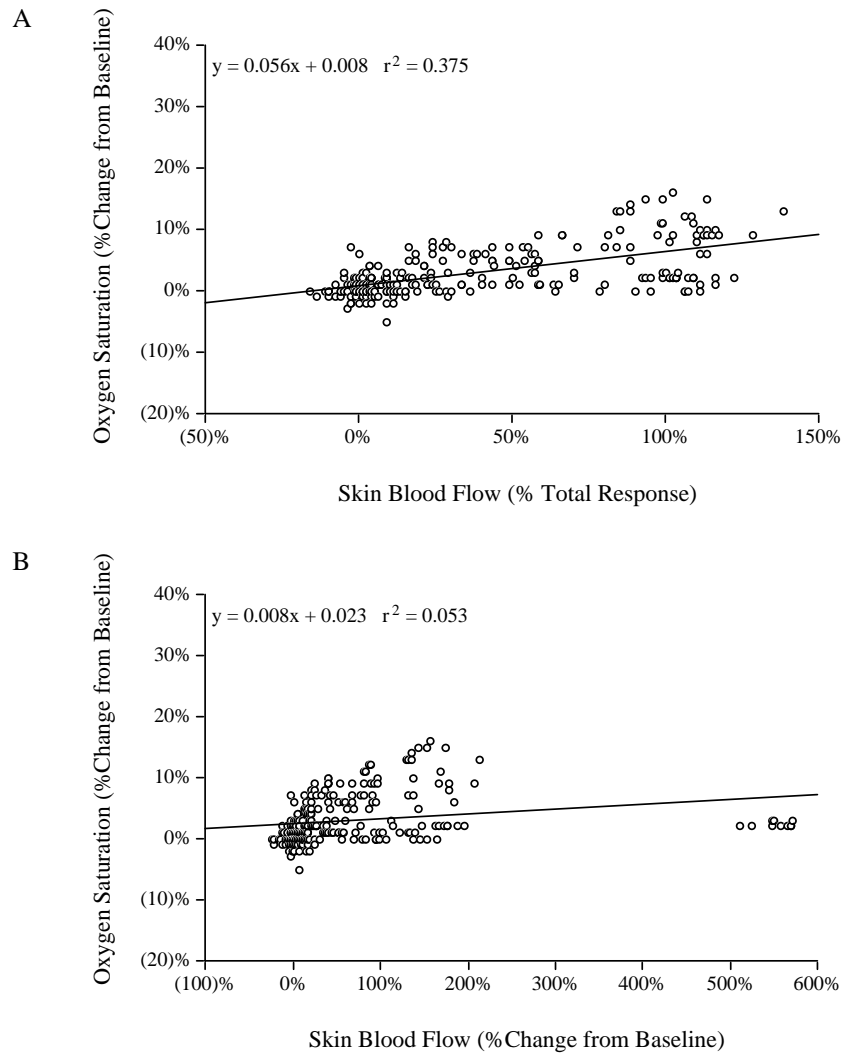
		%Baseline Perfusion	% Response Perfusion	% Baseline CMBC	% Response CMBC
%StO <sub>2</sub>	r	0.23*	0.61*	0.09	0.53*
	r <sup>2</sup>	0.05	0.38	0.01	0.28
%Hct	r	0.48*	0.54*	0.48*	0.50*
	r <sup>2</sup>	0.23	0.29	0.23	0.25
%HbT	r	0.39*	0.81*	0.24*	0.72*
	r <sup>2</sup>	0.15	0.66	0.06	0.52
%HbO <sub>2</sub>	r	0.51*	0.81*	0.34*	0.72*
	r <sup>2</sup>	0.26	0.66	0.11	0.52
%Hb	r	-0.63*	-0.62*	-0.45*	-0.55*
	r <sup>2</sup>	0.39	0.38	0.20	0.31

## Discussion

The primary finding of this investigation was that NIRS-derived variables increased with elevated skin blood flow stimulated by direct local heating. This finding was not totally unexpected since light for the NIRS system must pass through the skin, and some absorbance in the capillaries and venules of the skin would be anticipated. Under resting conditions, the impact of skin blood flow on NIRS derived measurements would be expected to be magnified as the skin temperature increased. At this time, NIRS cannot differentiate between the sources of increased absorbance, whether it is from skin or muscle.

We chose this model to test our hypotheses because previous investigators have shown that muscle does not play an active role in thermoregulatory responses. Johnson, et al. [Johnson, 1976] showed that local heating of the skin to 42.5° C resulted in an increase in muscle temperature but not increase in muscle blood flow. Forearm muscle blood flow,

measured by  $^{125}\text{I}$ -labeled antipyrine clearance, was unchanged during 50 minutes of continuous warming. Detry, et al. [Detry, 1972] made a similar observation, which was substantiated by the finding that muscle blood flow was increased only after the onset of forearm exercise. In contrast, elevation of skin temperature to 43-44° C raises skin blood flow to near maximal levels over the course of 40-60 minutes [Johnson, 1976; Taylor, 1984].

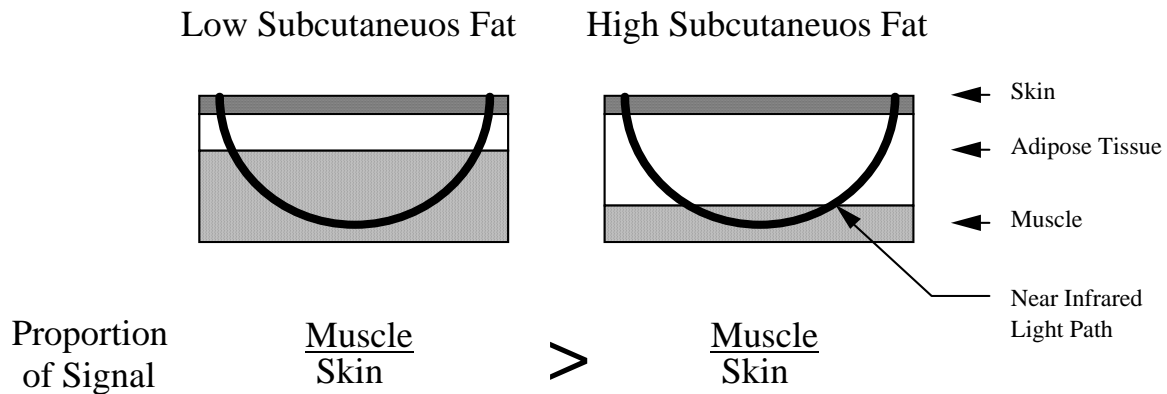


**Figure 5. The relationship across subjects between LDV- and NIRS-derived variables improved in almost all cases when the LDV variable was expressed relative to the total observed response (Panel A) rather than to the baseline observed at 30 °C water bath temperature (Panel B).**

The findings of the current study contradict those of Mancini et al. [Mancini, 1994] who observed no change in NIRS-derived index of desaturation following local heating of the arm. The authors did not provide details of their subject population (skin and subcutaneous fat thickness), so it is difficult to determine whether this had a specific

effect on their measurements. Further, the spacing between the light source and the sensor was not described, but the authors stated that the probe was designed for a photon depth of 2-3 cm. Assuming that the depth of penetration was half the distance between the light source and the detector [Homma, 1996], then the separation must have been between 4 and 6 cm. The separation used in the current investigation was almost half that and, therefore, was more likely to have been influenced to a greater degree by skin blood flow as proportionally more of the NIRS light-path is associated with skin rather than muscle tissue.

Near infrared light must pass through subcutaneous fat and skin tissue in order to reach the muscle of interest and, therefore, these tissues may affect the ability of the NIRS signal to describe changes in muscle blood flow. Adipose tissue is metabolically inactive relative to muscle and, therefore, the NIRS signal is likely to be diluted when sampling an individual with large amounts of subcutaneous fat. That is, during protocols that alter muscle oxygen saturation and blood flow, a lack of change in these variables in adipose tissue would be “averaged” with the changes in muscle tissue such that the total observed change by NIRS would be attenuated. In a lean individual, the near infrared light would be more likely to penetrate to the depth of muscle tissue such that changes in oxygen saturation in the muscle would represent a larger proportion of the signal (Figure 6).



**Figure 6. The relative proportion of muscle to skin that is sampled by NIRS is greater in a lean individual than in an individual with a greater amount of subcutaneous body fat.**

The ability to focus measurements at specific tissue layers requires the choice of the appropriate probe or sensor spacing. To decrease the relative contribution of skin blood flow to the total NIRS signal, wider sensor spacing and, therefore, greater tissue depth might be employed. In this particular project, only one separation was used (25 mm) in all subjects, who had different amounts of skin and subcutaneous fat. Interestingly, review of the correlation data within individual subjects suggests that those subjects with a smaller skinfold thickness demonstrated smaller changes in the NIRS-derived variables. Additionally, in these individuals the relationships between LDV- and NIRS-derived variables also were generally lower. For example, the correlation coefficient describing the relationship between the percent of the total response in skin blood perfusion and the percent change in total hemoglobin was greater in those subjects with higher skinfold thickness ( $r^2=0.67$ ,  $n=4$ , skinfold  $\geq 8\text{mm}$ ) than in those with lower skinfold thickness

( $r^2=0.15$ ;  $n=3$ , skinfold  $\leq 5$  mm). Therefore, when using a single probe with a set distance between the light source and the detector, the likelihood that skin blood flow changes will be reflected in the modification in the NIRS-derived variables will be related directly to the amount of skin and subcutaneous fat overlying the muscle of interest.

Homma et al. [Homma, 1996] suggested that adjusting the distance between the light source and the detector may be required to compensate for different amounts of adipose tissue across subjects. They observed that in subjects with greater amounts of adipose tissue NIRS detected little desaturation with exercise when using a shorter distance between the light source and detector (20 mm). When measurements were made with a probe with a greater distance (30 mm), all subjects exhibited deoxygenation. To prevent such issues, many NIRS studies have selected subjects with a low percentage of body fat [McCully, 2000]. This would not be practical in the clinical application of this technique, but McCully et al. [McCully, 1997] observed that adequate NIRS signals were obtained when subjects had a body mass index of less than 32. However, the influence of overlying tissue on the total NIRS signal may limit the comparisons between subjects of different adiposity and within subjects when attempting to study the NIRS signal during experimental protocols, such as before and after chronic exercise training, which alter the amount of subcutaneous fat.

In this study, the NIRS-derived hematocrit increased during the heating procedure. CMBC (derived by LDV) has been suggested as an index of hematocrit in skin blood flow and also was observed to increase during local skin heating. Assuming that the majority of the signal for the increased NIRS-derived hematocrit was caused by increased hematocrit in the microcirculation of the skin, these findings are not unexpected. That is, vasodilation in the skin is likely to reduce accumulation of erythrocytes on the arterial side of the capillary beds and small vessels [Gaethgens, 1984]. Hemoglobin within erythrocytes would not be expected to increase as a result of local heating, and therefore increasing total hemoglobin is likely to reflect a total accumulation of erythrocytes. Similarly, since oxygen demand would not increase during local heating, the elevated NIRS signal for oxyhemoglobin and decreasing signal for deoxyhemoglobin are likely the result of an increase in the number of erythrocytes within the skin vasculature as a consequence of vasodilation.

## Limitations

One of the primary limitations of this study is that the temperature applied to the skin by the circulating water was not directly at the site of measurement. The measurement probes passed through the water bladder and were affixed to the skin with holders, which provided some insulation from the warm water. This became apparent visually when the water bladder was removed at the completion of the test. The skin directly underneath the bladder was flushed, but the area underneath the probes was not. The probe holders were cut down as much as possible, but were required to maintain the position of the probes. These visual observations were supported by the skin temperatures measured directly at the measurement sites during the first phase of the study. However, we were able to induce changes in skin blood flow despite this limitation, and therefore the observed

changes in the NIRS derived variables may have been further exaggerated had we been able to attain higher skin temperatures.

The results of this study may be complimented by future investigations in which increased skin vasodilation above non-exercising muscles is stimulated by an increased core temperature. Core temperature has been suggested to have a 20-fold greater influence on skin blood flow than skin temperature [Rowell, 1986]. While making laser Doppler and NIRS measurements in the leg, core temperature could be raised by heating of the upper torso. Similarly, measurements might be made in the arm or upper body while performing lower body exercise. Also, other perturbations of local skin blood, such as drug iontophoresis, may further elucidate this relationship.

A second limitation of this study is that the results may be applicable only to the measurement of skin and muscle in the resting condition. Although skin blood flow would increase during exercise in response to elevated core temperature, blood flow to an exercising muscle would be anticipated to increase by as much as 50 times [Laughlin, 1985]. Therefore, the relative contribution of skin blood flow to the total signal from NIRS placed above an exercising muscle would be expected to decrease as exercise intensity and the consequent muscle blood flow increased.

## **Conclusions**

Local heating of the thigh during supine rest resulted in marked skin vasodilation, which was associated with concomitant changes in the hemoglobin and hematocrit derived from NIRS measurement. Further studies are required to determine the optimal spacing between the light source and the photodetector to minimize the effect of skin vasodilation relative to skinfold thickness. Also, the influence of skin blood flow on NIRS-derived variables during exercise should be investigated.

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